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Activity of Ciprofloxacin Against Methicillin-Resistant *Staphylococcus aureus*

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Ciprofloxacin, a carboxy quinolone antibiotic with a broad spectrum of activity, was tested against 54 strains of methicillin-resistant *Staphylococcus aureus*. The ciprofloxacin MICs for 50 and 90% of the isolates were 0.25 and 0.5 μg/ml, respectively, and its MBC for 90% of the isolates was 1.0 μg/ml. Killing kinetic studies were conducted in vitro with ciprofloxacin and vancomycin individually and in combination. The results of these studies showed that ciprofloxacin at 2 μg/ml and vancomycin at 10 μg/ml decreased the number of organisms by approximately 1.5 $\log_{10}$ after 6 h. The combination of ciprofloxacin plus vancomycin did not alter the rate of killing over that achieved by ciprofloxacin alone. The in vitro killing of resistant staphylococci was rapid, and the potential use of ciprofloxacin for infections caused by methicillin-resistant *S. aureus* should be further explored.

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are becoming increasingly prevalent (15). Therapies with cephalosporins and macrolides have failed in such infections (1, 13). At present, the only generally accepted, effective therapy is vancomycin (12, 14). Unfortunately, this antibiotic has a few side effects, such as anaphylaxis, nephrotoxicity, and ototoxicity (8).

Ciprofloxacin, a member of the carboxy quinolone family of antibiotics, has been shown to have a wide spectrum of activity for both gram-positive and gram-negative organisms (2, 4, 7, 16). Although this antimicrobial agent cannot achieve very high levels in serum or tissue when given orally (information on file, Miles Laboratories, West Haven, Conn.), such therapy has proven effective in the treatment of gram-negative bacillary infections of various body sites (D. Felmingham, R. A. Wall, G. L. Ridway, and R. N. Gruneberg, Program Abstracts Intersci. Conf. Antimicrob. Agents Chemother. 24th, Washington D.C., abstr. no. 398, 1984; B. E. Scully, K. Jules, and H. C. Neu, Program Abstracts Intersci. Conf. Antimicrob. Agents Chemother. 24th, Washington D.C., abstr. no. 850, 1984). Hence, ciprofloxacin was examined for its in vitro activity against MRSA.

MATERIALS AND METHODS

Antibiotics. Antibiotic powders were obtained from the respective distributors as follows: ciprofloxacin, Miles Laboratories, West Haven, Conn.; clindamycin, The Upjohn Co., Kalamazoo, Mich.; gentamicin, Schering Corp., Kenilworth, N.J.; oxacillin and methicillin, Bristol Laboratories, Syracuse, N.Y.; nafcillin, Wyeth Laboratories, Philadelphia, Pa.; and vancomycin, Eli Lilly & Co., Indianapolis, Ind. All were used according to the instructions of the manufacturers.

Bacterial strains. Isolates of MRSA were obtained from the clinical microbiology laboratory of the Veterans Administration Medical Center, East Orange, N.J. All isolates were obtained from different patients and were kept frozen at −70°C and subcultured onto blood agar plates (BBL Microbiology Systems, Cockeysville, Md.) before testing. *S. aureus* ATCC 29213 was included as a control strain for susceptibility testing.

Determinations of MICs and MBCs. MICs were determined by macrobroth dilution in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) (11). For the determination of gentamicin susceptibilities, Mueller-Hinton broth was supplemented with 25 mg of Mg^{2+} and 50 mg of Ca^{2+} per liter. An equal volume of organisms from a 4-h broth culture was added to serial twofold dilutions of antibiotics to yield a final bacterial density of 5×10^5 CFU/ml. The organisms were inoculated at the surface of the liquid medium. The tubes were gently mixed by hand and incubated in a forced-air incubator at 35°C for 16 to 20 h. The MIC was defined as the lowest concentration of antibiotic that completely inhibited growth. After the MIC determination was made, the tubes were vortexed and reincubated. After 4 h, 0.1 ml of broth from each visually clear tube was surface streaked onto a 1-1/2-inch (3.81-cm) square of a Mueller-Hinton agar plate (BBL). Plates were incubated for 16 to 24 h at 35°C, and the number of visible colonies was recorded. The MBC was recorded as the lowest concentration of antibiotic that produced killing of the initial inoculum of 99.9% or greater.

Time-kill experiments. The rate of killing of MRSA by ciprofloxacin and vancomycin individually and in combination was determined for 15 randomly selected strains. The concentrations tested included 1, 2, and 5 μg of ciprofloxacin per ml, 2 and 10 μg of vancomycin per ml, and 2 and 5 μg of ciprofloxacin per ml plus either 2 or 10 μg of vancomycin per ml. To determine whether synergistic killing occurred, we studied the two antibiotics at one-half the MBC of each antibiotic individually and in combination. The final density of organisms in 5 ml of antibiotic-containing Mueller-Hinton broth and a control broth with no antibiotics was either 5×10^5 or 5×10^7 CFU/ml. Portions were removed at 0, 6, 24, and 48 h after inoculation, and the number of viable organisms was quantitated by serial 10-fold dilutions and by subcultures of 0.1-ml portions onto blood agar plates.

RESULTS

The results of susceptibility testing for the 54 isolates of MRSA are shown in Table 1. All isolates were resistant to

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TABLE 1. MICs and MBCs for 54 strains of MRSA

Antimicrobial agent	MIC (μg/ml)			MBC (μg/ml)		
	Range	50%	90%	Range	50%	90%
Ciprofloxacin	0.25–0.5	0.25	0.5	0.25–1.0	0.5	1.0
Vancomycin	0.5–1.0	1.0	1.0	0.5–4.0	1.0	2.0
Gentamicin	≤0.25–>16	2.0	>16	0.5–>16	2.0	>16
Clindamycin	0.12–>16	0.5	>16	4.0–>16	>25	>25
Methicillin	12.5–>50	>50	>50	50–>50	>50	>50
Nafcillin	3.1–>25	>25	>25	6.2–>25	>25	>25
Oxacillin	3.1–>25	>25	>25	25–>25	>25	>25

the three penicillinase-resistant penicillins, and many were also resistant to gentamicin. Although 45% of the isolates were resistant to clindamycin, all isolates were susceptible to ciprofloxacin and vancomycin. Ciprofloxacin and vancomycin were bactericidal for all strains tested, MBCs for 90% of the isolates (MBC_{90}s) being 1.0 and 2.0 μg/ml, respectively. The results of studies with ciprofloxacin and vancomycin in combination were similar for the 15 strains tested, and this combination showed no advantage over ciprofloxacin alone.

The results of the killing kinetic studies are shown in Fig. 1 and 2. When an inoculum of 5×10^5 CFU/ml was used, the rate of bacterial killing did not differ significantly between ciprofloxacin (Fig. 1A) and vancomycin (Fig. 1B). When an inoculum of 5×10^7 CFU/ml was used, some strains appeared to regrow by 24 h of incubation with vancomycin at 2 μg/ml (twice the MIC; obtained by using an inoculum of 5×10^5 CFU/ml), and this accounted for the spread in colony counts seen at 24 and 48 h of incubation (Fig. 1B and 2A). At a higher concentration of vancomycin (10 μg/ml, or 5 to 10 times the MIC), killing was effective even up to 48 h. With ciprofloxacin at 1 μg/ml (two to four times the MIC), the organisms did not regrow, but the killing effect ceased after 24 h of incubation (Fig. 1A). After 6 h of incubation, a 1.5- to 2-log decrease and a 3-log decrease (after 24 h) in the number of CFU/ml was noted for both ciprofloxacin at 2 μg/ml and vancomycin at 10 μg/ml. Unlike vancomycin, ciprofloxacin showed no concentration-dependent killing of the bacteria at observations of up to 24 h (Fig. 1B). The combination of vancomycin plus ciprofloxacin was unable to alter the killing rate over that of ciprofloxacin alone (Fig. 2). Only a 2-log decrease in the number of viable organisms was noted after 6 h, and a 3.5-log decrease was observed after 24 h. Furthermore, when these two antibiotics were used at one-half the MBC, alone and in combination, neither synergistic nor antagonistic bacterial killing was shown (Fig. 2B).

DISCUSSION

The incidence of infections caused by MRSA is on the rise. The number of available antibiotics with proven efficacy for treating infections caused by these organisms has not been similarly expanded. Therapeutic trails with cephalosporins and the newest beta-lactam antibiotics, such as the carbapenems, have met with similar clinical failures despite good in vitro results (1; A. Berry and G. Archer, Program Abstracts Intersci. Conf. Antimicrob. Agents Chemother. 24th, Washington, D.C., abstr. no. 342, 1984). Gentamicin cannot be used for at least two reasons: the incidence of gentamicin resistance among the staphylococci may be already as high as 20%, and gentamicin resistance develops easily in these organisms when it is used alone (10). For a similar reason, rifampin cannot be used for these organisms. In fact, rifampin resistance can still occur when used in combination with certain cell wall-active antibiotics (5a, 6).

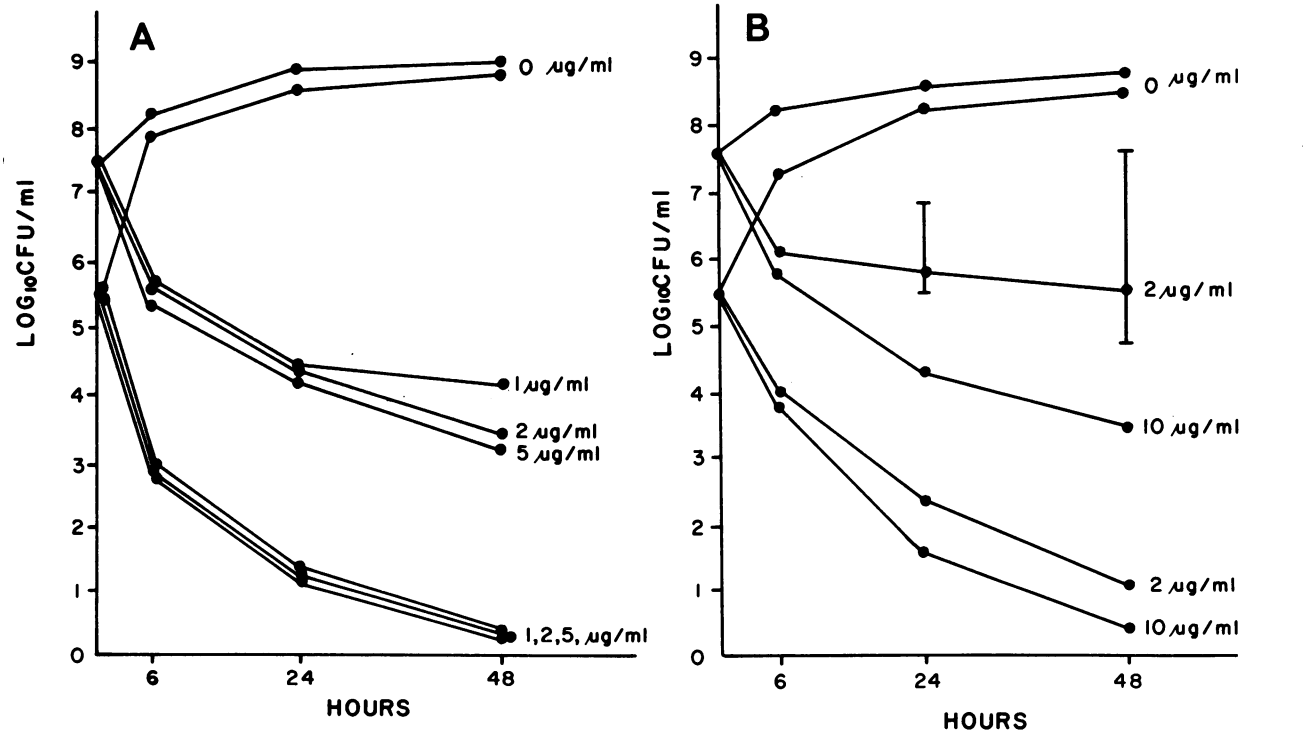

FIG. 1. Killing kinetics of ciprofloxacin (A) and vancomycin (B) for MRSA at increasing concentrations of the antibiotics. Inocula of 5×10^5 and 5×10^7 CFU/ml were tested. The amounts of variation in the data points are ±0.5 log except where the amount of variation is otherwise indicated.

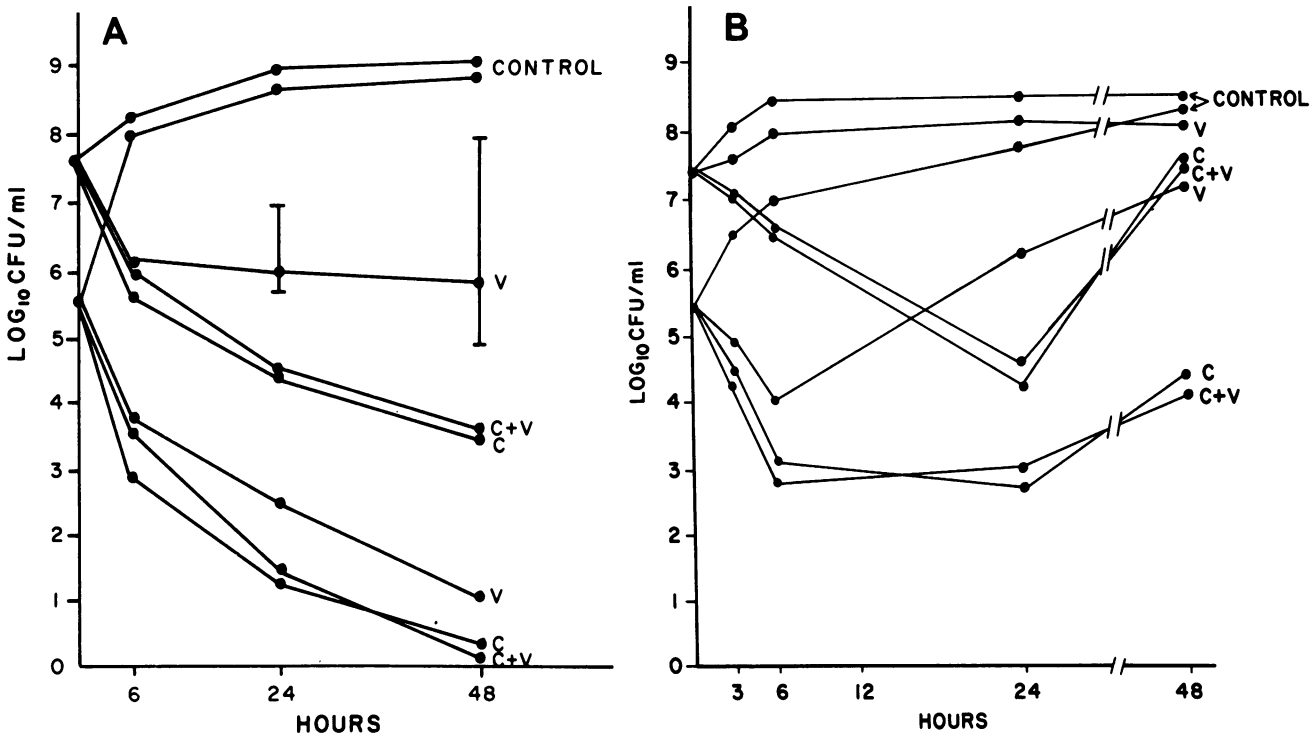

FIG. 2. Killing kinetics of MRSA by vancomycin (V) and ciprofloxacin (C), individually and in combination, at a concentration of 2 μg/ml each (A). (B) The two antibiotics at one-half the MBC, used individually and in combination.

This organism can also be resistant to the macrolides, such as erythromycin, and even if staphylococci are initially susceptible, resistance to erythromycin has been known to develop during therapy of serious infections (13). The prospect for successfully treating MRSA infections with clindamycin is similarly unpromising, as 45% of such isolates in our hospital are resistant to clindamycin. Vancomycin remains as the single acceptable antibiotic for the therapy of infections caused by these organisms.

Ciprofloxacin is an orally absorbable antibiotic which has been reported to attain concentrations of 4 to 6 μg/ml in serum (information on file, Miles Laboratories). As a first impression, such a concentrations in serum seem too low to effectively treat staphylococcal infections. However, the in vitro susceptibility results of 54 strains of MRSA show that the MBC_{90} is 1.0 μg/ml. Hence, the peak concentration in serum is 6 to 12 times the MCBs of these organisms. In comparison with nafcillin for methicillin-susceptible *S. aureus*, nafcillin attains concentrations of approximately 10 μg/ml in serum, and its MBC_{90} is 2 μg/ml (9). Nafcillin, then, can attain only five times the MBC_{90} for methicillin-susceptible *S. aureus*. Yet nafcillin has been quite successful in the therapy of infections caused by methicillin-susceptible *S. aureus*, including endocarditis, and is recommended as initial therapy (3). Success in the treatment of endocarditis depends on multiple factors, of which achievable antibiotic concentration in serum is only one (5). Therefore, oral ciprofloxacin has a potential to be effective therapy for infections caused by MRSA, including endocarditis.

The results of killing kinetic studies illustrate at least four points concerning ciprofloxacin. (i) At an inoculum of 5×10^5 CFU/ml, no differences in killing kinetics could be discerned between ciprofloxacin and vancomycin at the several concentrations studied. (ii) At the higher inoculum of 5×10^7 CFU/ml, vancomycin killing at 2 μg/ml (2 times the MIC) was considerably slower than vancomycin at 10 μg/ml (5 to 10 times the MIC) and, in fact, allowed for organism regrowth at 24 h. Ciprofloxacin at similar multiples of the MIC prevented regrowth of the organism, but its killing action stopped after 24 h. Hence, ciprofloxacin appeared to show an advantage over vancomycin at low multiples of the MIC. (iii) Ciprofloxacin at 2 μg/ml killed as effectively and as rapidly as did vancomycin at 10 μg/ml. (iv) Combining vancomycin with ciprofloxacin produced neither synergism nor antagonism against these organisms.

In conclusion, oral ciprofloxacin has the potential to become an effective agent for the therapy of MRSA infections. Both the susceptibility results and the killing kinetic studies showed that ciprofloxacin may have the potential to be the most effective agent for difficult-to-treat infections caused by these organisms. Because of previous experiences with a divergence of in vitro and clinical results with cephalosporins, enthusiasm for ciprofloxacin should be restrained. It should be noted that ciprofloxacin belongs to a different class of antibiotics and has a different mechanism of action than that of the beta-lactams, which often fail to kill MRSA. The final determination of whether ciprofloxacin will prove effective for the treatment of infections caused by MRSA in humans must await the results of clinical trials.

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